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MEMORANDUM

SUBJECT: Review of the Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in Rats (ARGUS 1416-003)

FROM: Gary Kimmel
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TO: Annie Jarabek
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This is a review of the Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in Rats (Protocol 1416-003, Part D) carried out by Argus Research Laboratories, Inc. (Audited Final Report, 1 June 2000). The materials in the package included the audited draft final report, and appendices A-H (Appendix D, Certificate of Analysis, is "to be supplied at a later date."). The results as presented in the report are assumed to be accurate. My copy of the final report and appendix H, Quality Assurance, were not signed. The raw data was generally reviewed, but was not used to recalculate the findings reported in the summary tables.

Study Design and Animal Husbandry:

The study design generally follows the guidance of the EPA/OPPTS guidelines for a developmental toxicity study. The report cites the draft guidelines of January 1996; the final guidelines were published in August 1998. Several points that require clarification with Argus:

- I assume the missing consumption data (1-2 points in many of the groups, Tables B1 & B2) did not affect the results. This was not noted in the report.

- The dosage concentrations for the week of 14 Feb 00 - 20 Feb 00 were "based on water consumption data from the previous week because the latter data were not available... ." This week would have been the first week of gestation, since the "cohabitation period" was from 14 Feb - 23 Feb 00. The Argus report indicates "This deviation did not affect the outcome... ." I am not sure how they can support this, especially since this is the period when preimplantation effects would be initiated.

- Breeder males were also exposed. I assume that the females were only bred with males exposed to the same exposure concentration. This is not stated anywhere.

- Appendix E: Environmental and Husbandry
  page 227 - There was a maximum temperature of 100.0 F. There were 8 points out-of -range. page 228 indicates the 100.0 readings were "false temperature recordings as indicated by facility deviation." It is not clear what is meant by this, whether this includes the three 84F readings, and how they justify saying that these deviations did not adversely affect the outcome or interpretation of the study.

There were 140 female rats delivered to Argus, 121 used in the study. Each exposure group was comprised of 24 females, assigned on a random basis, stratified by weight. There was no maternal death. Of these females, 20 were selected for evaluation. Of these, 19, 19, 17, 20 and 20, were pregnant in the 0, 0.01, 0.1, 1 and 30 mg/kg/day groups, respectively. The EPA/OPPTS guidelines recommend 20 pregnant animals per group at necropsy. Thus, the power of the study to detect an exposure-related response is somewhat lower than what would be achieved with the recommended number of animals.

Maternal Endpoints:

Three dams in the 30mg/kg/day group showed an increase in localized alopecia which was statistically significant. The report indicates the increase was not considered exposure-related, since "this observation is commonly observed in rats in the laboratory environment." This statement is not supported and makes little sense. None of the other groups showed any signs of alopecia, and Argus did not include this information for the historical controls; consequently, the "commonly observed" assertion is not supported. Moreover, if this is "commonly observed" to the extent that some importance cannot be assigned to a statistically significant finding in a study with less-than-optimal power, then the endpoint is of no value in the analysis. The alopecia was observed in the three females over 9-11 days during mid-late gestation; not just on a single day. This finding should be considered biologically significant and exposure-related.

There were no other the maternal parameters that were clearly supportive of exposure-related effects. There was a statistically significant increase in corrected maternal body weight gain over gestation in the 0.1 and 30 mg/kg/day groups, and an increase (not statistically significant) in the 1.0 mg/kg/day group. There was also a reduction (not statistically significant) in gravid uterine weight in three of the four exposure groups. These latter changes may be associated with a reduced number of implants in the exposed groups (see below).

On page 22, footnote "a" indicates that the number of corpora lutea in one animal was incorrectly recorded. However, Table B17 does not indicate this missing data. Argus needs to clarify this apparent inconsistency.

Developmental Endpoints:

Table B17 does not report preimplantation loss as an endpoint. There is an increase in this parameter over control (Group I = 12%; II = 18%; III = 20%; IV = 16%; V = 25%). Whether this is statistically or biologically significant is unclear; although there was a decrease in "live fetuses" in three of the four exposure groups, and this was statistically significant in the highest exposure level. Given the reduced power of this study to determine an effect, some attention should be paid to this finding. The lack of an effect on "live fetuses" at the 1.0 mg/kg/day level is not clear, and these results by themselves are insufficient to establish an effect level below 30 mg/kg/day. However, it would be prudent to consider the effect of percholorate on preimplantation loss and embryo/fetal viability in any other study reports that may be available.

The report summary notes that ossification sites per litter for sternal centers and forelimb phalanges were significantly reduced at 30 mg/kg/day, but that these were "considered reversible developmental delays." Developmental delays, be they permanent or reversible, are not discounted as potential indicators of developmental toxicity.

The report indicates that there was evaluation of the cartilage. Argus did not use the double-staining technique that is commonly used for staining bone and cartilage, nor do they indicate what alternate technique was used. Moreover, neither the report nor the historical data base (Appendix G) provide data on the cartilage assessment. This parameter would provide additional data that may be helpful in assessing the potential effect of the exposure on overall skeletal-cartilage development.

Summary:

Argus (p15) concluded that the maternal and fetal NOAELs are greater than 30.0 mg/kg/day, based on the lack of an exposure-related effect. However, based on my review of the data, there are signs of both maternal and developmental toxicity at 30.0 mg/kg/day.

Although alopecia may not be considered one of the most dramatic signs of maternal toxicity, it is recognized as a clinical sign. It occurred in this study over a period of time in three dams and was statistically significant. This finding may be an indicator that the 30 mg/kg/day exposure approximates the exposure level where maternal toxicity begins to deviate from background.

The signs of developmental toxicity include increased pre-implantation loss, decreased number of live fetuses, and a delay or alteration in ossification. These are all observed at the 30 mg/kg/day exposure level. The effects on viability may even occur at lower exposure levels, although this cannot be established with the results of this study.

None of these results are so clear cut that a definitive assessment of maternal and developmental toxicity can be made. However, the results are suggestive, and it is important that any potential maternal/developmental toxicity be considered in light of the overall data base on the toxic potential of perchlorate exposure.